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Current Opinion in Biotechnology

Genetic alphabet expansion biotechnology by creating unnatural base pairs

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Recent studies have made it possible to expand the genetic alphabet of DNA, which is originally composed of the four-letter alphabet with A–T and G–C pairs, by introducing an unnatural base pair (UBP). Several types of UBPs function as a third base pair in replication, transcription, and/or translation. Through the UBP formation, new components with different physicochemical properties from those of the natural ones can be introduced into nucleic acids and proteins site-specifically, providing their increased functionalities. Here, we describe the genetic alphabet expansion technology by focusing on three types of UBPs, which were recently applied to the creations of DNA aptamers that bind to proteins and cells and semi-synthetic organisms containing DNAs with a six-letter alphabet.

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Current Opinion in Biotechnology 2018, **51**:8–15

This review comes from a themed issue on **Nanobiotechnology**

Edited by **Alfonso Jaramillo** and **Mark Howarth**

http://dx.doi.org/10.1016/j.copbio.2017.09.006

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Introduction

The Watson-Crick base pairing with the four-letter alphabet is the key to the central dogma of all living organisms on Earth. The exclusive complementarity of the A–T(U) and G–C pairs makes possible DNA replication and RNA transcription by polymerase reactions and translation to proteins by codon-anticodon interactions in ribosome reactions. Conventional biotechnology always relies on this base-pairing rule with the four-letter alphabet, and in turn, the four-letter alphabet limits the further advancement of the technology. Therefore, the idea to expand the genetic alphabet is inevitable, which can be achieved by creating an artificial base pair (unnatural base pair, UBP) that functions as a third base pair in the replication, transcription, and translation (Figure 1).

Increasing the number of the genetic alphabet can enhance the information density and capacity of nucleic acids. With the enhanced information capacity using a six-letter alphabet, the present three-base codon table could be expanded from 64 (4^3) to 216 (6^3) combinations, and a huge number of new amino acids could be assigned in the expanded table for the incorporation into proteins by translation [1,2]. Alexander Rich who first suggested the initial concept of the genetic alphabet expansion indicated the possibility of a compressed two-base codon table using a six-letter alphabet, in which twenty standard amino acids can be assigned into 36 (6^2) combinations [3]. As a result, the increased numbers of the components (five or six nucleotide and more than 20 amino acid components) could greatly augment the biopolymer functionalities of proteins, as well as nucleic acids.

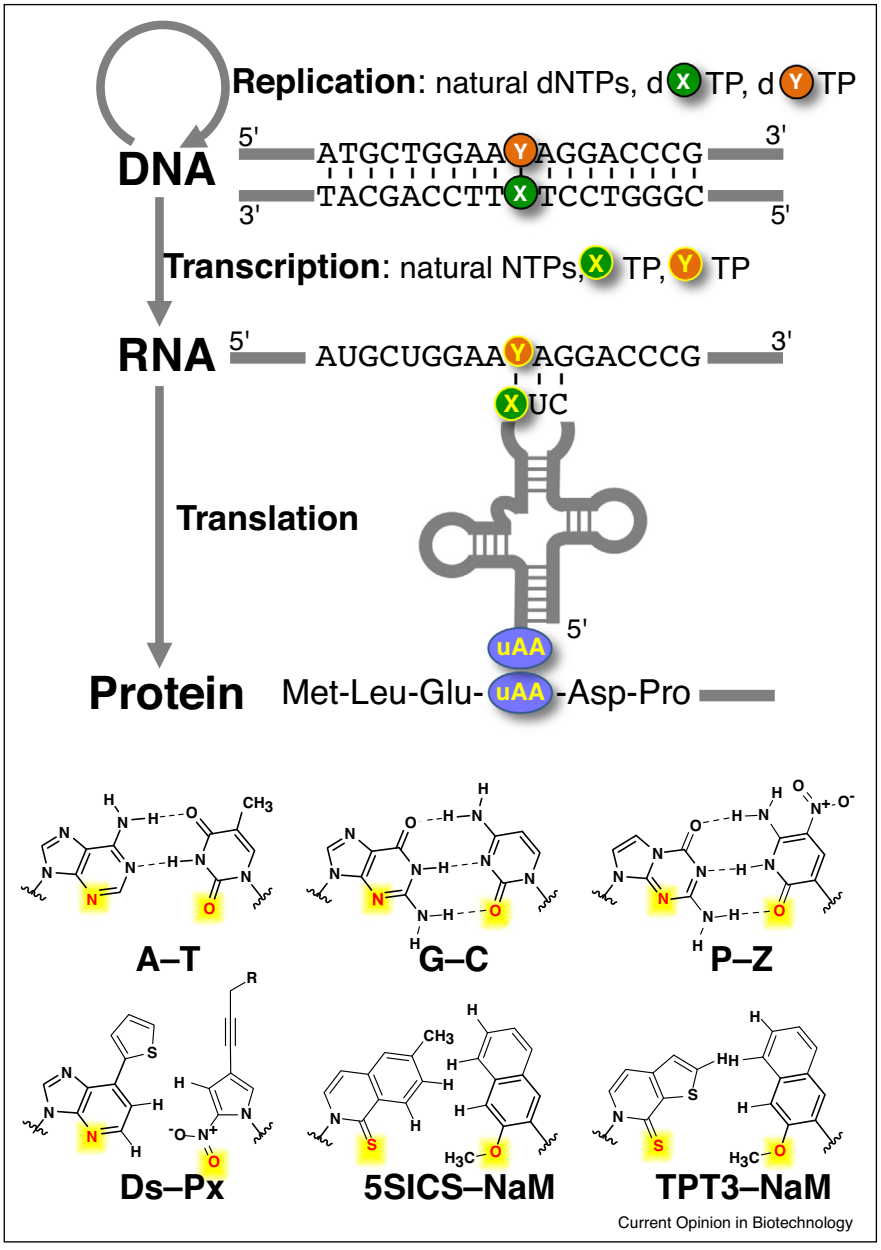
Recently, three groups independently created UBPs that exhibits high fidelity in replication and/or transcription and demonstrated various applications using the UBPs [4–7]. In this review, we introduce the genetic alphabet expansion technology by focusing on these UBPs and their applications to generating high-affinity DNA aptamers and *in vivo* systems with six-letter alphabet.

Creation of unnatural base pairs

In the past decade, three groups of Benner, Romesberg, and ourselves developed own UBPs with different ideas. Benner's group generated P–Z pair with a different hydrogen bonding donor and acceptor pattern [8–10] from those of the natural base pairs, by addressing the problems of their initial UBP, such as isoguanine and isocytosine pair [11]. In contrast, Romesberg's group chemically synthesized a series of hydrophobic base analogues based on their initial hydrophobic self-UBP [12], and generated several UBPs, such as 5SICS–NaM [13–15] and TPT3–NaM [16], that function in replication. We initially developed hydrogen-bonded UBPs by combining the concepts of the different hydrogen-bonded pattern and steric-hindrance exclusion [2,17]. Through continuous improvements, our group finally created the hydrophobic Ds–Px pair [18–20] by the concept of shape complementarity [21] with steric and electrostatic exclusions [2,17,22,23], to remove the mispairings with natural bases.

The common factor of all of these UBPs is that they have a proton donor atom in each UB at the minor groove side,

Figure 1



Overview of the genetic alphabet expansion employing an unnatural base pair (X–Y). The unnatural base pair (UBP) can provide a new biotechnology, enabling the site-specific incorporation of functional components of unnatural nucleotides and amino acids (uAA) into nucleic acids and proteins, respectively, according to the genetic information flow. At the bottom, chemical structures of the natural Watson-Crick base pairs and the representative UBPs are shown, where proton acceptors at the minor groove side for polymerase recognition are indicated in red.

like 2-nitrogen of purines and 2-keto of pyrimidines (atoms in red in Figure 1), to interact with polymerases [24,25]. Initially, DNA fragments containing UBs are chemically synthesized by a conventional phosphoramidite method. Then, the UBP-DNA fragments can be amplified by PCR using the natural and UB triphosphates.

These UBPs exhibit high selectivity in PCR amplification, in which each triphosphate concentration was adjusted to optimize the selectivity for each UBP. For example, the selectivity of the P–Z pair is 99.8% per theoretical PCR cycle by *Taq* DNA polymerase with 0.1 mM dATP, dGTP and dTTP, 0.4 mM dCTP, 0.05 mM dZTP, and 0.6 mM dPTP [10]. The selectivity of the TPT3–NaM pair is >99.98% per doubling by One*Taq* DNA polymerase with 0.2 mM natural dNTPs and 0.1 mM dTPT3TP and dNaMTP [16]. The selectivity of the Ds–Px pair is >99.97% per doubling by Deep

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